REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED
13.07.2004	Final Report	1.03.2002-30.09.2003

4. TITLE AND SUBTITLE Characterization of physical and chemical properties of marine bioadhesives from living organisms and hydrated biofilms.

5a. CONTRACT NUMBER

5b. GRANT NUMBER N00014-02-1-0331

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
J.A. Callow
R. Wetherbee
M.E. Callow

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Birmingham, Birmingham, B15 2TT, UK
University of Melbourne, Parkville, Victoria, Australia

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy St.
Arlington, VA222l7-5000

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AILABILITY STATEMENT
Distribution Unlimited **DISTRIBUTION STATEMENT A**
~~Approved for Public Release~~
Distribution Unlimited

13. SUPPLEMENTARY NOTES

20040722 051

14. ABSTRACT

The grant funded Dr. Wetherbee's sabbatical at the University of Birmingham. The results of th study show that diatoms stick more strongly to hydrophobic PDMS than a hydrophilic model surface like glass, and this is reflected in a greater interaction energy between individual adhesive strands and PDMS. Thus, these results correlate well with the field observation that PDMS foul-release coatings often fail to diatom slimes. As many antifouling coatings use hydrophobicity as a major deterrent to fouling, this strategy alone may not work for diatoms, particularly over time. Rather, this may be a preferred surface for these organisms and additional measures will be necessary to prevent their adhesion.

15. SUBJECT TERMS
Adhesives, diatoms, PDMS elastomer, foul-release, AFM, biofouling.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Prof. J.A. Callow
Unclass	Unclass	Unclass	Unlimited	6	19b. TELEPHONE NUMBER *(Include area code)* +44-121-414-5559

FINAL REPORT

GRANT NUMBER: **N00014-02-1-0331**

PRINCIPAL INVESTIGATORS: JA Callow[1], R Wetherbee[2], ME Callow[1]

INSTITUTIONS: [1]University of Birmingham, [2]University of Melbourne

GRANT TITLE: Characterization of Physical and Chemical Properties of Marine Bioadhesives from Living Organisms and Hydrated Biofilms.

AWARD PERIOD: 1 March 2002-30 September 2003

OBJECTIVES:
To quantify the adhesive and elastic properties of diatom bioadhesives in native, hydrated form, and on a range of different substrata relevant to Navy needs.

APPROACH: The majority of the research on the grant was conducted during the course of a sabbatical year to be spent by Dr Wetherbee at Birmingham University. Hydrodynamic assays using a calibrated, turbulent flow cell were used to compare adhesion strength of a range of diatom species on model test surfaces of different wettability, viz. acid washed glass and a silicone elastomer (PDMSE, T2 silastic supplied by Dr A B Brennan, University of Florida). The response of several species of diatoms to these surfaces was observed using time-lapse video microscopy and their cell movements (i.e., settlement, reorientation and motility) observed and measured. Environmental Scanning Electron Microscope (ESEM) was used to make direct observations of live, hydrated diatom adhesives, trails and biofilms. Chemical force microscopy on diatom adhesive strands used cantilevers functionalised with PDMS monolayers.

ACCOMPLISHMENTS: Dr Wetherbee's sabbatical was divided into 2, 6-month blocks, and for personal reasons, the second 6 months were taken at the University of Melbourne, while other experiments continued at Birmingham.

Comparative whole cell adhesion assays were successfully undertaken on contrasting model surfaces (glass and PDMSE) using the diatoms *Amphora coffeaeformis* var. *perpusilla*, *Navicula perminuta* and *Crapsedostauros australis*.

The same diatoms were observed under the ESEM at the University of Birmingham after having settled for varying periods of time. We imaged diatom cells and their associated mucilages, plus attempted to image diatom trails after an hour of settlement. Biofilms were imaged after settled cells had been allowed to glide for up to 2 days. The thin, hydrated trails were not visualized with this technique, so this work ceased.

Diatom cells were filmed as they settled onto hydrophilic and hydrophobic surfaces and the patterns of their settlement, reorientation and gliding movement recorded and compared over time.

In collaboration with Dr Michael Higgins and Prof. Suzi Jarvis (who provided access to an Asylum AFM at Trinity College, Dublin), we performed chemical force microscopy on diatom adhesive strands using cantilevers functionalised with PDMS monolayers (provided by Dr Gilbert Walker (University of Pittsburgh).

CONCLUSIONS:

1) All 3 diatoms adhered more strongly to the hydrophobic PDMSE than the hydrophilic, glass surface, as judged by the shear stress needed to remove 50% of attached cells(Figs, 1 and 2). However, there were distinct inter-specific differences, especially on PDMSE. The shear stress needed to remove 50% of attached *Craspedostaurus* and *Amphora* cells from PDMSE was between 20-30 Pa (Fig. 2). However, attached cells of *Navicula* could not be removed in the flow cell and the much higher forces provided by the water jet apparatus were necessary to remove the adhered cells of this species.

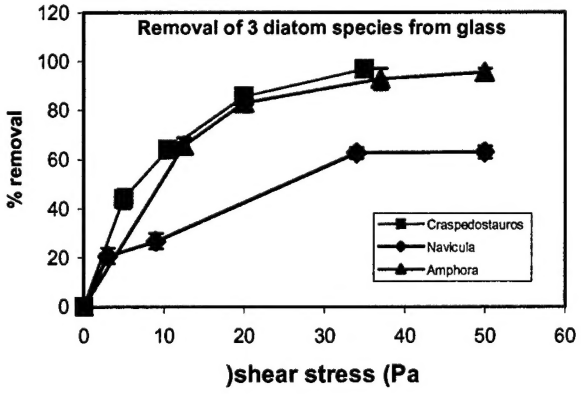

Figure 1. *Removal of diatom species from glass*

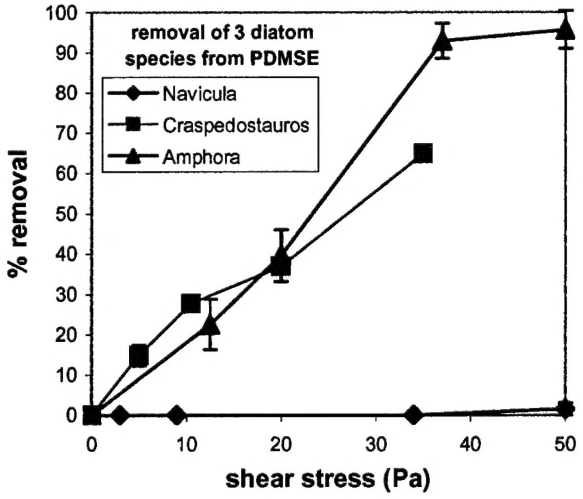

Figure 2 *Removal of diatom species from T2 Silastic PDMSE*

2) The development of adhesion strength of *Amphora* to PDMSE is time-dependent, but not on glass (Fig.3). *Navicula* may show a similar trend but the experiment needs to be repeated. The situation with *Craspedostauros* appears to be different with no progressive increase in strength of attachment with time (data not shown).

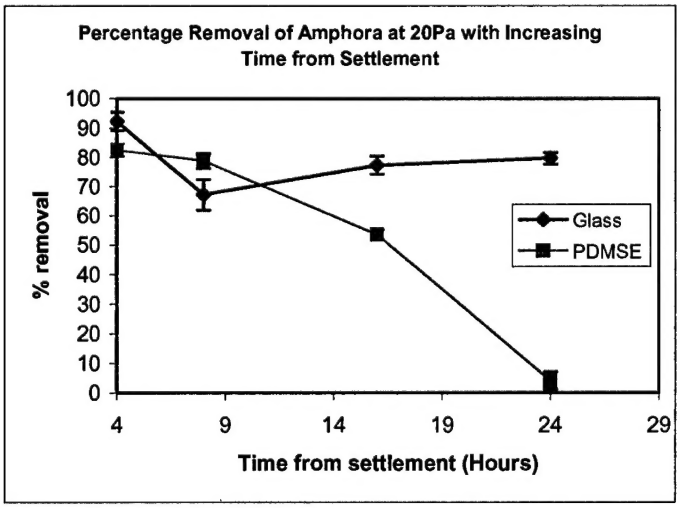

Figure 3 Increased adhesion strength of Amphora with time on T2 Silastic PDMSE but not on glass

3)Time-lapse video microscopy of diatom settling, reorientation and gliding was observed for several diatoms on both hydrophilic and hydrophobic surfaces. In all species studied, initial adhesion was observed, and cells reorientated onto a raphe and were presumably capable of gliding. Given time, cells eventually move equally well on glass and PDMSE. However, in the first 30 mins of being placed on the surfaces, cell movement varies between species. For *Amphora* movement was greater on PDMSE than glass, for *Navicula* movement was greater on glass, while for *C. australis* movement was the same on both surfaces. There are several ways of interpreting these results, but overall we suggest that motility is not a good indicator of adhesion to surfaces.

4)ESEM was useful in showing the outer coating of individual cells and thick biofilms. However, these layers were highly hydrated and it was not possible to observe much structure because of the excess water present. The mucilages change from being over-saturated with water to dehydrated after only a small change in humidity, and this affect is instantly reversible. The level of humidity needed to induce this change varies considerably for the mucilages of different diatom species.

5) AFM cantilevers coated with PDMS monolayers were used to measure the adhesive and elastic properties of the adhesive strands of the diatoms *C. australis*, *N. perminuta* and *Haslea* sp. and compared with standard, hydrophilic silicon nitride tips. The technique of 'fly-fishing' was used to study the interaction forces with single (or few) adhesive strands. Results are still to be fully processed for all species but one generalisation is possible. In experiments conducted with standard tips, analysis of force curves from several species gave interaction energies of the order 100 pN (modelled for a single strand) which is similar to values previously reported. However, in most experiments with PDMS-coated tips S-shaped force curves were observed which result from the continual bridging of the adhesive strands between the cantilever tip and cell surface during successive extension-retraction cycles. This demonstrates a greater interaction energy between the adhesive strands and PDMS but we cannot say how much greater because release does not occur. However, the result is consistent with the whole cell adhesion studies that show that these diatoms adhere more strongly to PDMS than hydrophilic glass. We are now completing these studies using the three different diatom species that were used in the flow chamber adhesion assays shown above.

SIGNIFICANCE: The results of this study show that diatoms stick more strongly to hydrophobic PDMS than a hydrophilic model surface like glass, and this is reflected in a greater interaction energy between individual adhesive strands and PDMS. Thus, these results correlate well with the field observation that PDMS foul-release coatings often fail to diatom slimes. As many antifouling coatings use hydrophobicity as a major deterrent to fouling, this strategy alone

may not work for diatoms, particularly over time. Rather, this may be a preferred surface for these organisms and additional measures will be necessary to prevent their adhesion.

PUBLICATIONS AND ABSTRACTS:

Two papers are in preparation and will be submitted in 2004:

Understanding the interaction between biofouling diatoms species and silicone elastomers: I. adhesion and motility.

Understanding the interaction between biofouling diatoms species and silicone elastomers: II. Use of chemical force microscopy to measure interaction energies between adhesive strands and PDMS substrates.